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INTERACTION OF ELECTROMAGNETIC FIELDS WITH CHONDROCYTES IN GEL CULTURE

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Alan J. Grodzinsky, Sc.D.
Michael D. Buschmann, Ph.D.
Yehezkiel A. Gluzband, M.S.

Department of Electrical Engineering and Computer Science
Massachusetts Institute of Technology
Cambridge, MA 02139

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19. ABSTRACT: **The specific objectives of this research period were: (1) to quantify the effect of applied electric fields on chondrocyte metabolism, using a range of stimulation frequencies and amplitudes; (2) to compare the chondrocyte biosynthetic response to applied fields at early times in agarose gel culture before an extracellular matrix has accumulated and at later times after significant deposition of matrix around and between the cells; and (3) to begin to interpret the biosynthetic response to applied fields in terms of models of physical mechanisms. The results of these studies suggest that electric fields applied to chondrocytes in agarose can modulate the synthesis of proteoglycans and protein constituents. Biosynthesis may be inhibited or stimulated depending on the amplitude of the applied current density. In addition, the presence of extracellular matrix may enhance the ability of normal chondrocytes and cells in intact cartilage to respond to electric fields, although the presence of matrix was not required for the stimulatory response to be observed with Swarm rat chondrosarcoma cells.**

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SUMMARY

The interaction of electromagnetic fields with biological tissues is of increasing importance from the standpoint of potential health hazards and of possible beneficial (e.g., diagnostic and therapeutic) effects. To address these issues, the research accomplished during this period focused on the biosynthesis of highly specialized protein and polysaccharide molecules by mammalian connective tissue cells. Since the synthesis and processing of these macromolecules is one of the most important biological functions of cartilage cells, altered matrix metabolism is known to be a very sensitive indicator of the effectiveness of an environmental stimulus, and of the pathway by which the stimulus evokes a response.

The long-term goal is to study long-term (chronic) and short-term (acute) effects of electromagnetic field exposure on the synthesis and turnover of highly charged proteoglycan (PG) molecules and their glycosaminoglycan (GAG) constituents, collagens, and other non-collagenous proteins by normal cartilage cells (chondrocytes) extracted from articular cartilage, and in rat chondrosarcoma cells (a continuous cell line).

To address these goals, the specific objectives of this research period were: (1) to quantify the effect of applied electric fields on chondrocyte metabolism, using a range of stimulation frequencies and amplitudes; (2) to compare the chondrocyte biosynthetic response to applied fields at early times in agarose gel culture before an extracellular matrix has accumulated and at later times after significant deposition of matrix around and between the cells; and (3) to begin to interpret the biosynthetic response to applied fields in terms of models of physical mechanisms.

The results of these studies suggest that: (1) both normal chondrocytes and Swarm rat chondrosarcoma cells in agarose culture can, under proper culture conditions, continue to synthesize matrix macromolecules at a rate similar to that in native cartilage, and (2) electric fields applied to chondrocytes in agarose can modulate the synthesis of proteoglycans and protein constituents. Biosynthesis may be inhibited or stimulated depending on the amplitude of the applied current density. In addition, the presence of extracellular matrix may enhance the ability of normal chondrocytes and cells in intact cartilage to respond to electric fields, although the presence of matrix was not required for the stimulatory response to be observed with Swarm rat chondrosarcoma cells.

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INTERACTION OF ELECTROMAGNETIC FIELDS WITH CHONDROCYTES IN GEL CULTURE

INTRODUCTION

The interaction of electromagnetic fields with biological tissues is of increasing importance from the standpoint of potential health hazards and of possible beneficial (e.g., diagnostic and therapeutic) effects. While much of the literature to date has focused on experimental observation and verification of putative field effects, increasing attention is now being paid to the physical mechanisms that may underlie such electromagnetic interactions. There is still little understanding of basic physical mechanisms or of the biochemical pathways whose disturbance is essential for interaction to occur.

Electromagnetic interactions with biological tissues may involve several different physical and biochemical processes. Sufficiently intense radiation can result in significant damage to cells by thermal processes alone [1]. The effects of subthermal processes have been harder to quantify. A recent theoretical model [2] suggests that weak electric fields as small in amplitude as that associated with thermal noise may still be detected by cells. The long-term goal of our research is aimed at identifying the mechanisms of normal cell functioning that are altered by electromagnetic fields.

To address these issues, we have focused on the biosynthesis of highly specialized protein and polysaccharide molecules by cartilage, with and without exposure to the combined effects of electrical and chemical perturbations. Since the synthesis and processing of these matrix macromolecules is one of the most important biological functions of connective tissue cells, altered matrix metabolism is known to be a most sensitive indicator of the effectiveness of an environmental stimulus, and of the pathway by which the stimulus evokes a response.

In particular, we have studied the effect of electromagnetic fields on the synthesis and turnover of highly charged proteoglycan (PG) molecules and their glycosaminoglycan (GAG) constituents, collagens, and other non-collagenous proteins

by normal cartilage cells (chondrocytes) extracted from articular cartilage, and in rat chondrosarcoma cells (a continuous cell line). The overt gene expression of chondrocytes results in the synthesis and deposition of a structurally integrated extracellular matrix composed of these macromolecules [3]. The use of cartilage cells is motivated, in part, by the fact that these cells are naturally subjected to endogenous electric fields over a 5-6 decade frequency range at current densities as high as 1 mA/cm^2, produced by *in vivo* mechanical loading [4,5].

To examine these biosynthetic responses, it is essential to use cell populations that are as homogeneous as possible. Because cartilage cells vary with depth in the tissue and along a joint surface, it is advantageous to either extract homogeneous subpopulations or to uniformly mix all extracted cells from the tissue, and culture them in a medium in which applied field parameters can be precisely controlled. For this and other important reasons, we chose to culture and test the cells in agarose gel discs. Such culture techniques have been well-established for normal chondrocytes and chondrosarcoma cells. In addition, the chondrocyte-containing agarose gels are transparent. Therefore, an array of assays can eventually enable additional characterization of single cell responses, including staining and cytofluorescence techniques that have already been developed elsewhere. In addition, the agarose/cell system enables us to couple our experimental methods and results to the theoretical models of interaction mechanisms recently developed in our laboratory [6-11] and by other investigators [12].

The specific objectives for the funded 6-month study were, therefore, (1) to quantify the effect of applied electric fields on chondrocyte metabolism, using a range of stimulation frequencies and amplitudes; (2) to compare the chondrocyte biosynthetic response to electric fields at early times in agarose culture before accumulation of an extracellular matrix and at later times after significant deposition of matrix around and between cells; and (3) to begin to interpret the biosynthetic response to applied fields in terms of models of physical mechanisms.

BACKGROUND

Cartilage Structure and Composition

Articular cartilage is the dense, skeletal connective tissue that functions as a bearing material in synovial joints. Cartilage from adult animals and humans is avascular, aneural, and alymphatic. The tissue's sparse population of cells (chondrocytes) derives its nutrition primarily from the synovial fluid in the joint cavity [13]. The chondrocytes are responsible for the synthesis, maintenance, and gradual turnover of an extracellular matrix composed primarily of hydrated type II collagen fibrils and highly charged proteoglycan molecules [14] which together account for 20-30% of tissue wet weight. These matrix macromolecules are shown schematically in Fig. 1. The proteoglycan constituents contain a high net charge at physiological pH and thereby exert significant electrostatic swelling forces that help to maintain tissue hydration and help to resist mechanical compression.

Effects of Physical Forces on Cartilage Matrix Metabolism

Physiologic mechanical compression can induce a variety of electrical, mechanical and chemical stimuli capable of affecting chondrocyte metabolism. Investigators from several laboratories have consistently found that static compression of cartilage explants decreases the rate of synthesis of proteoglycans and proteins [15,16]. The mechanism for this inhibition appears to be associated with the decrease in tissue water content and the concomitant decrease in intratissue (extracellular) pH produced by static compression [17].

Recent studies [16] have also shown that dynamic compression of cartilage above a threshold loading frequency under physiological conditions can stimulate matrix biosynthesis. Dynamic compression is also known to produce pressure gradients and fluid flow within the extracellular matrix. *This fluid flow generates electrical potentials and currents known as streaming potentials and currents. (Fig. 1)* The past few years has seen significant progress in: (1) the experimental verification that

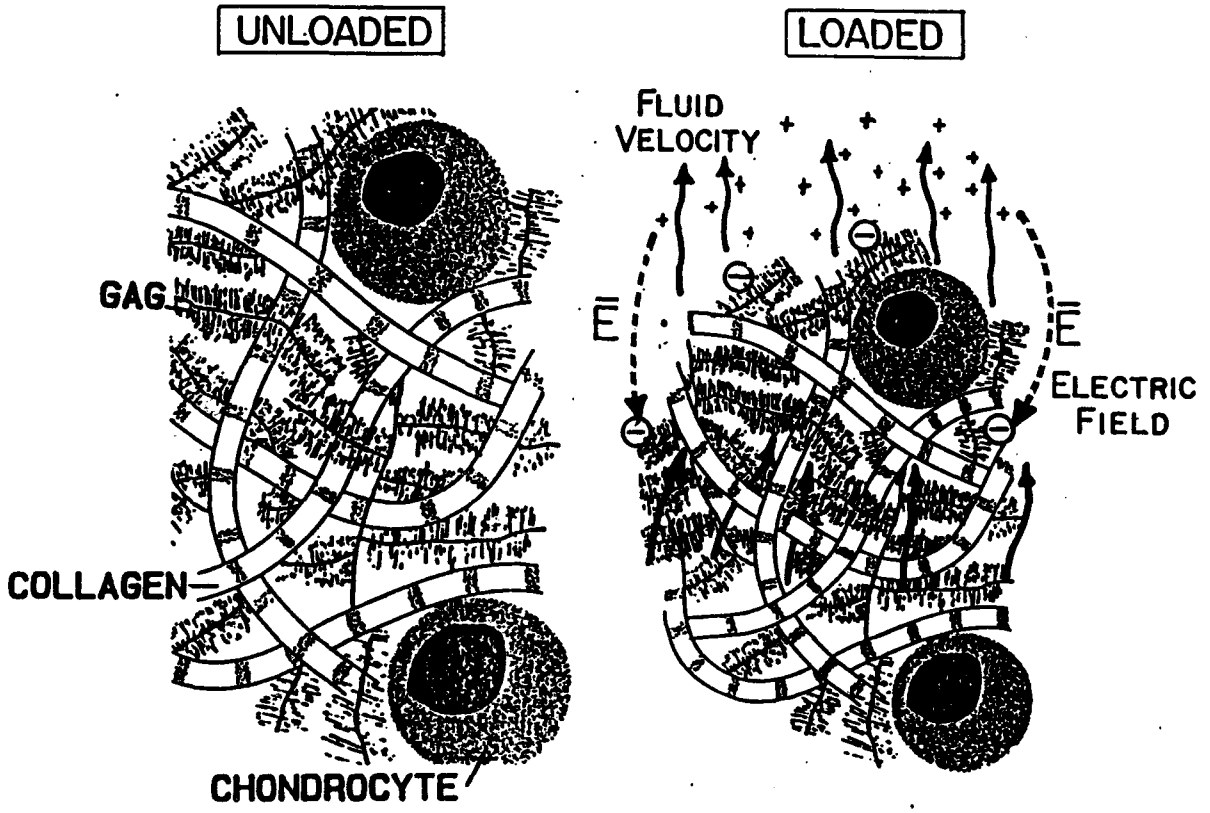
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Figure 1

an electrokinetic mechanism is responsible for these endogenous electrical signals in cartilage [4,5]; (2) the demonstration that fluid convection of ions past the carboxyl and sulfate fixed charge groups of the proteoglycans are responsible for streaming potentials [18]; and (3) the quantification of the amplitude and frequency response of streaming potentials and streaming currents produced in living cartilage organ culture explants by physiological levels of oscillatory mechanical compression [6], and the verification of theoretical models for electrokinetic transduction in cartilage matrix at the macroscopic [5,19] and molecular [20] levels.

Cartilage is now seen to be one of the most electrically active tissues that does not contain excitable cells. The possibility that endogenous electrical currents generated by mechanical loads may play a role in cellular response has been hypothesized in soft and hard connective tissues [21]. In order to study this important possibility in cartilage, investigators have stressed the importance of quantifying the effects of applied fields on the synthesis and turnover of proteoglycans and collagen. Thus, it is well accepted that the quantity and quality of the proteoglycans and proteins synthesized by chondrocytes are crucial and sensitive markers of the ability of an environmental stimulus to affect chondrocyte behavior.

Our previous experimental work on the response of mammalian cells to electromagnetic fields focused on the effect of applied electric currents on the synthesis of stress response and other proteins in intact organ culture explants of cartilage. The results of those studies showed that current densities below 30 mA/cm^2 at frequencies less than 1 kHz did not appear to stimulate synthesis of stress proteins [22,23]. However, total protein synthesis appeared to increase with increasing applied current density.

Need for Homogeneous Cell Populations

The fact that the total protein synthesis only appeared to increase with relatively high current densities may be due to the heterogeneity of the cell population within the cartilage explant. It is known that cell morphology and biosynthetic

response vary with position in the tissue. Therefore, isolation and mixing of the chondrocytes into agarose disks would minimize disk-to-disk variations. In addition, several groups have used a continuous cell line (Swarm rat chondrosarcoma) for biosynthesis studies, because of the demonstrated homogeneity of the response. Kimura et al. [24] have found that the proteoglycans and collagens synthesized by these chondrosarcoma cells are strikingly similar to those of native cartilage.

Chondrocytes Cultured in Agarose Gel

Previous studies have shown that chondrocytes cultured in agarose gel preserve their phenotypic expression as evidenced by the synthesis of cartilage-specific type-II collagen and proteoglycans [3]. Sun et al studied the proliferation and size of Swarm rat chondrosarcoma cells in agarose culture [24]. Aydelotte et al. [25] found that the agarose system was an ideal tool for quantifying proteoglycan synthesis and catabolism by means of sulfate radiolabel incorporation and chromatographic analyses of molecules remaining within and lost from the gels. Benya et al. used agarose culture to measure the effect of cytoskeletal modifications on reexpression of chondrocyte phenotype after treatment with retinoic acid [26].

In our own previous research that led to the present study, we that chondrocytes extracted from native cartilage could synthesize and accumulate a normal, cartilage-like extracellular matrix in agarose gel culture. We characterized the extent and the time evolution of chondrocyte proliferation, synthesis of GAG and proteins, loss of GAG, and total deposition of GAG-containing matrix within agarose gels during long term culture. To assess whether the matrix deposited within the agarose gel was mechanically functional, we measured several important mechanical and electromechanical properties of the agarose/chondrocyte disks at selected times during long-term culture: equilibrium elastic modulus, dynamic stiffness, and streaming potential induced by oscillatory mechanical compression. The results of these studies suggested that (1) both normal chondrocytes and Swarm rat chondrosarcoma cells in agarose culture could, under proper culture conditions, continue to synthesize matrix

macromolecules at a rate similar to that in native cartilage, and (2) chondrocytes in agarose could successfully mediate the assembly and accumulation of a normal, mechanically functional extracellular matrix.

METHODS: ELECTRICAL STIMULATION OF CHONDROCYTE/AGAROSE DISKS

Articular cartilage from the femoropatellar groove from 1 to 2-week-old calves was harvested as previously described [16] and incubated in DMEM supplemented with 12.5 mM Hepes, 0.1 mM nonessential amino acids, 0.4 mM L-proline, 10% FBS, 50 μg/ml ascorbate, and 0.1% penicillin/streptomycin changed daily at 5% CO_2, 37°C. One week later cells were extracted by sequential pronase and collagenase digestion [27]. In addition, Swarm rat chondrocytes were prepared for incorporation into agarose gels as previously described [24].

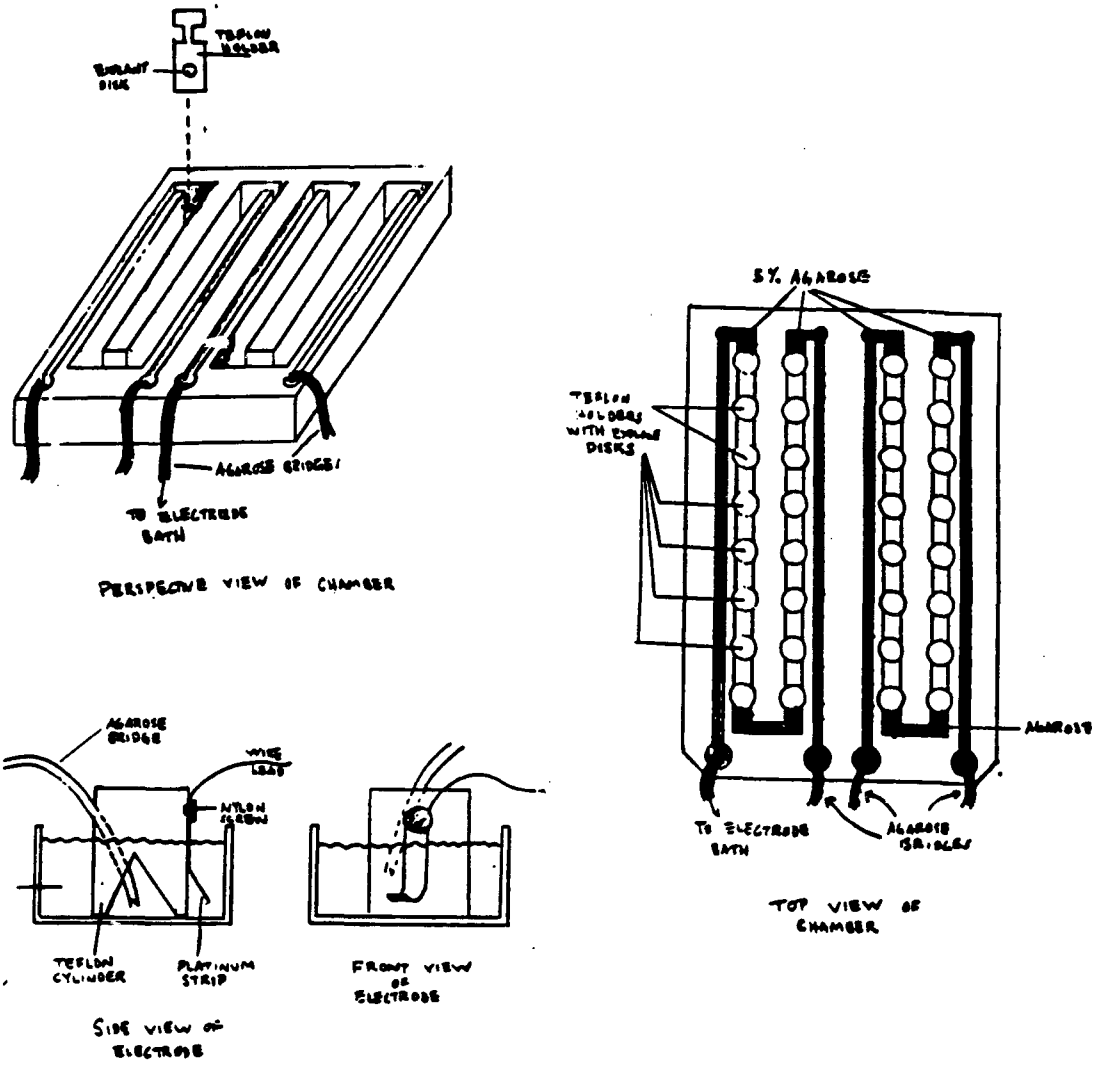
Chondrocytes were mixed into media containing 2% (w:v) low melting temperature (FMC) SeaPlaque agarose [24] at $\sim 2 \times 10^7$cells/ml and cast at 37°C between slab gel electrophoresis plates separated by 1-mm-thick Teflon spacers. After gelling at 4°C for 2 h, 16-mm-diameter by 1-mm-thick disks were cored from the gel. The chondrocyte/agarose disks were subsequently cultured on top of nylon mesh (to promote nutrient diffusion from below). Media (as above) was changed daily and analyzed for GAG content by dimethylmethylene blue (DMB) dye binding [28]. Control disks without chondrocytes were prepared and maintained in the same manner.

Electrical stimulation experiments were performed using disks from day 32 of a 70-day culture, and from day 6 and day 45 of a separate 47-day culture. Thus, one of the objectives was to test the effect of applied electric fields on chondrocyte biosynthesis at early times in culture (e.g., day 6) before a significant amount of extracellular matrix had accumulated, versus cells at later times (e.g., day 45) when a dense pericellular and interterritorial matrix had already developed. This comparison would enable us to explore the possibility that cell-matrix interactions could be

significant in regulating the effects of applied fields.

In each experiment, 48 3-mm diameter disks were cored from 4 larger 16-mm diameter disks and placed in cylindrically confining teflon holders in feed media 20 hours prior to stimulation. 4 groups of 12 disks each were then placed in electrical stimulation chambers in feed media containing 10 μCi/ml [^{35}S]sulfate and 20 μCi/ml [5-^{3}H]proline to assess proteoglycan and protein synthesis, respectively. Current density amplitudes of 0, 1, 10 and 30 mA/cm^2 at a frequency of 100 Hz were applied for 24 hours (12 disks at each current density). Disks were then removed from the chambers and washed 6 times over a 1-hr period to remove free label. The specimens were then digested with papain; aliquots of the digest were analyzed for DNA as a measure of cell number (by Hoechst 33258 [29]), glycosaminoglycan content [28] as a measure of total proteoglycan content, and sulfate and proline incorporation via liquid scintillation counting.

The teflon exposure chambers are shown schematically in Fig. 2, and have been described in detail previously [30,31]. Each chamber contains two independent lanes capable of holding 16 cylindrical agarose/chondrocyte disks. Gel disks were placed into teflon disk holders which, in turn, were inserted into the lanes. The disk holders accept 3mm diameter by 1mm thick disks. The lanes in the chambers were connected to platinum electrodes by means of "salt bridges" comprised of autoclavible plastic tubing filled with 5% w/v agarose. The electrode baths were filled with saline. This external electrode arrangement isolated the agarose/chondrocyte disks from and electrode reaction products. (Our previous experiments using cartilage organ culture explants showed that such electrode reaction products could themselves stimulate a stress response in the cartilage, as manifested by the synthesis of stress response proteins [23]). Previous tests of this system showed that negligible ohmic heating occured for current densities of interest.



Electrical chambers and electrodes used to apply current to explant disks. This figure depicts only one chamber and electrode.

Figure 2

RESULTS and DISCUSSION

Normal Bovine Chondrocytes

Figures 3-5 show the effect of applied electric fields on the biosynthetic response of primary cells (normal chondrocytes freshly extracted from cartilage tissue). Fig. 3 shows radiolabel incorporation for a group of disks at day 32 of culture, and Fig. 4 at day 45 of a separate culture. Fig. 5 shows radiolabel incorporation for disks taken at day 6 from the same culture as that of Fig. 4. Both proline and sulfate incorporation tended to decrease with increasing current densities up to 10 mA/cm^2 at both early and late times in culture, suggesting a decrease in proteoglycan and general protein synthesis. At higher current densities (Fig. 3 and 4), radiolabel incorporation appeared to be stimulated above that of control at late times in culture, after the development of a substantial extracellular matrix. This stimulatory response was absent in disks taken at 6 days, before the accumulation of matrix; here, application of 30 mA/cm^2 inhibited biosynthesis, as did 1 and 10 mA/cm^2.

This later result can also be compared to our previous experiments using disks of intact calf femoropatellar groove cartilage [22,23]. In those tests, protein synthesis was increased by current densities as high as 30 mA/cm^2. The intact tissue contains a very dense matrix with a proteoglycan content approximately 3-4 times higher than that of the late-time cultures of Fig. 3 and 4. While more tests are needed to confirm this hypothesis, it appears possible that the presence of extracellular matrix facilitates the stimulatory biosynthetic response of chondrocytes to sinusoidal fields.

Swarm Rat Chondrosarcoma Cells

The above experiments using intact cartilage explants or agarose/chondrocyte disks prepared from the same calf cartilage (e.g., Figs 3-5) involve somewhat inhomogeneous cell populations. The agarose disks are made from a well mixed population and, therefore, are much less subject to disk-to-disk variations, compared to the tissue disks. Nevertheless, there may be subpopulations within each agarose disks that are

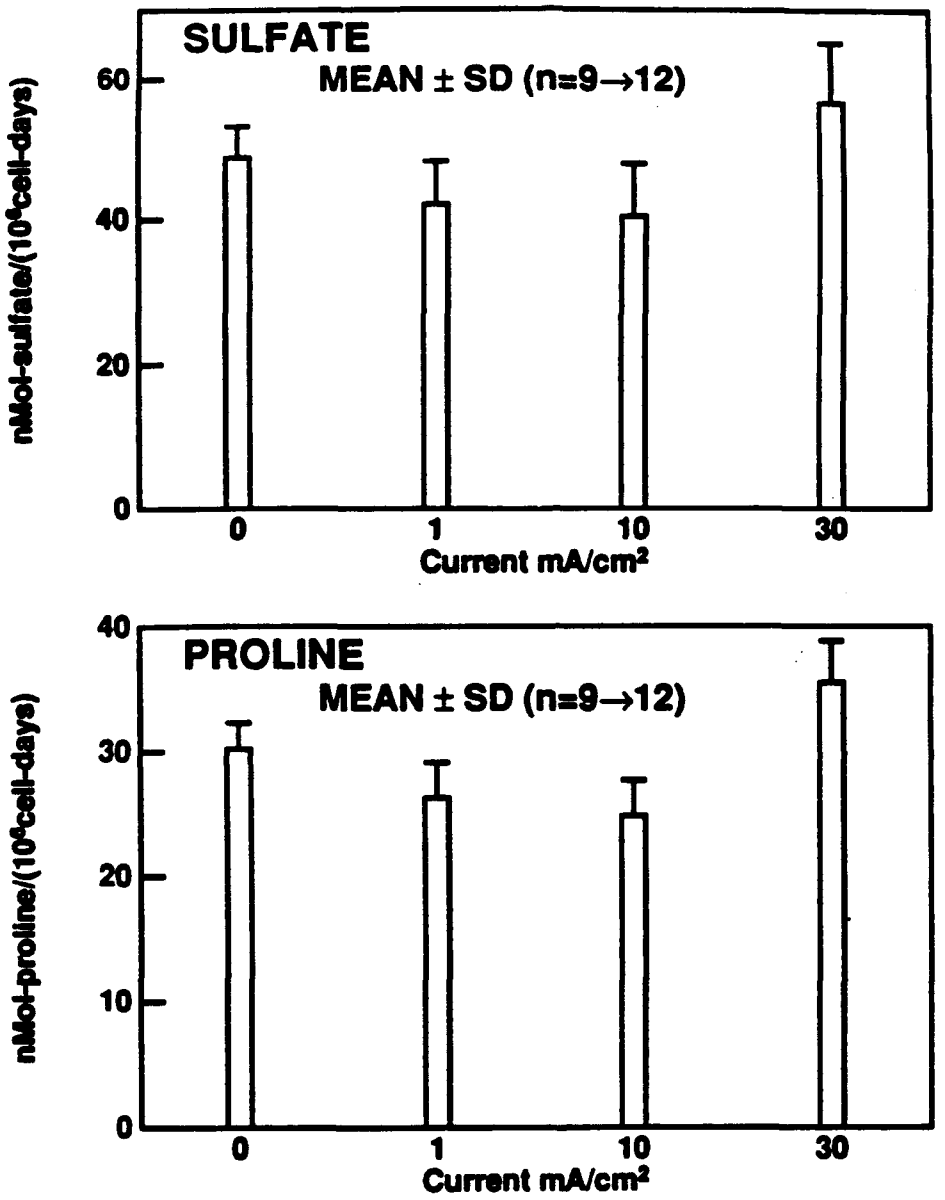


Figure 3 Groups of 12 3mm chondrocyte/agarose disks were subjected to current densities at 100Hz in the presence of radiolabeled sulfate and proline precursors. 2% agarose disks containing chondrocytes were taken on day 32 of the 70 day culture described in Chapter II and the currents were applied for 24 hours. All the bars at non-zero current densities are significantly different from the control group with no applied current, $p < 0.05$ using the two-tailed t-test.

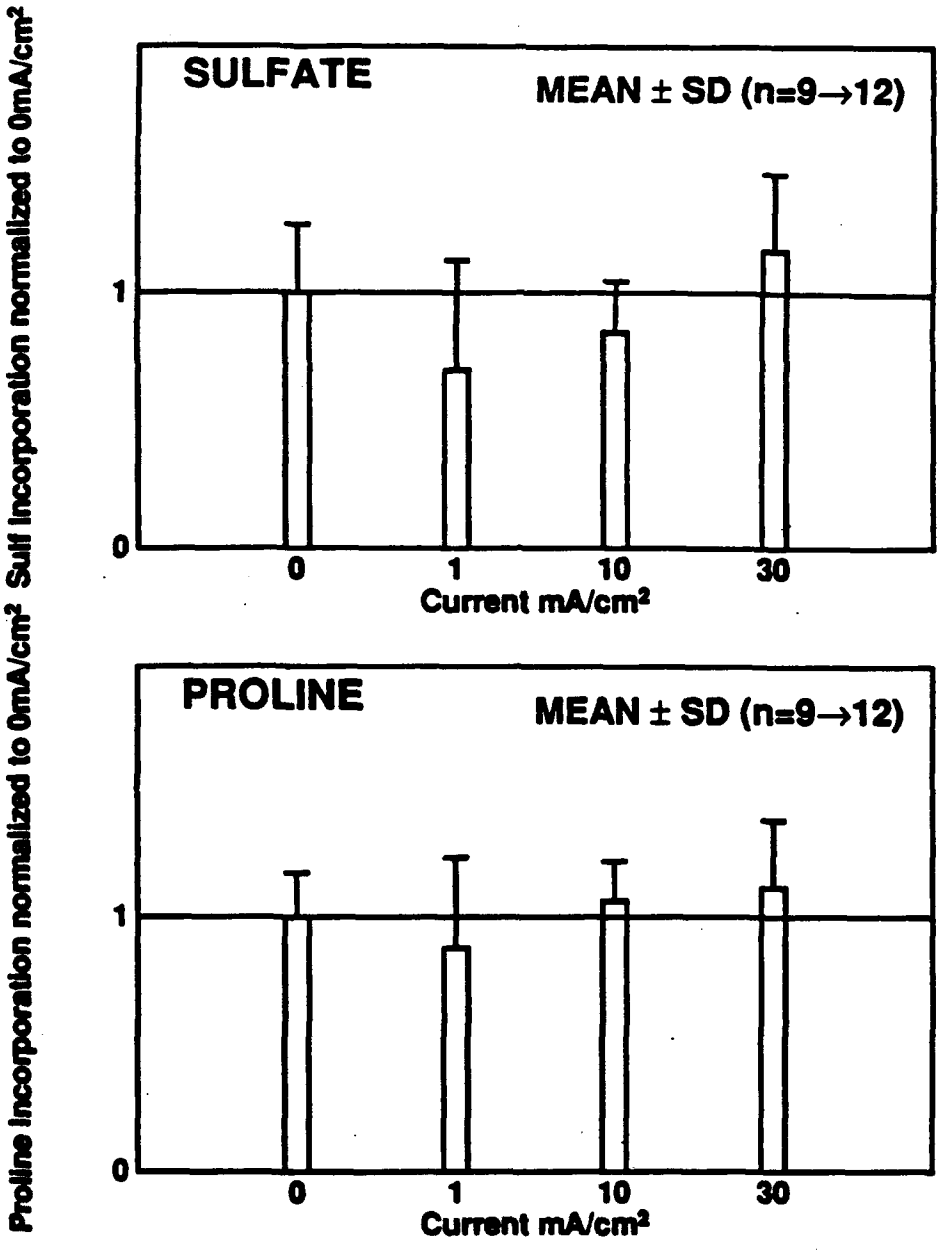


Figure 4 Groups of 12 3mm chondrocyte/agarose disks were subjected to current densities at 100Hz in the presence of radiolabeled sulfate and proline precursors. 1/2% agarose disks containing chondrocytes were taken on day 45 of the 47 day culture described in Chapter IV and the currents were applied for 24 hours. Only the bar at 1mA/cm² for sulfate is significantly different from the control group with no applied current, $p < 0.05$ using the two-tailed t-test.

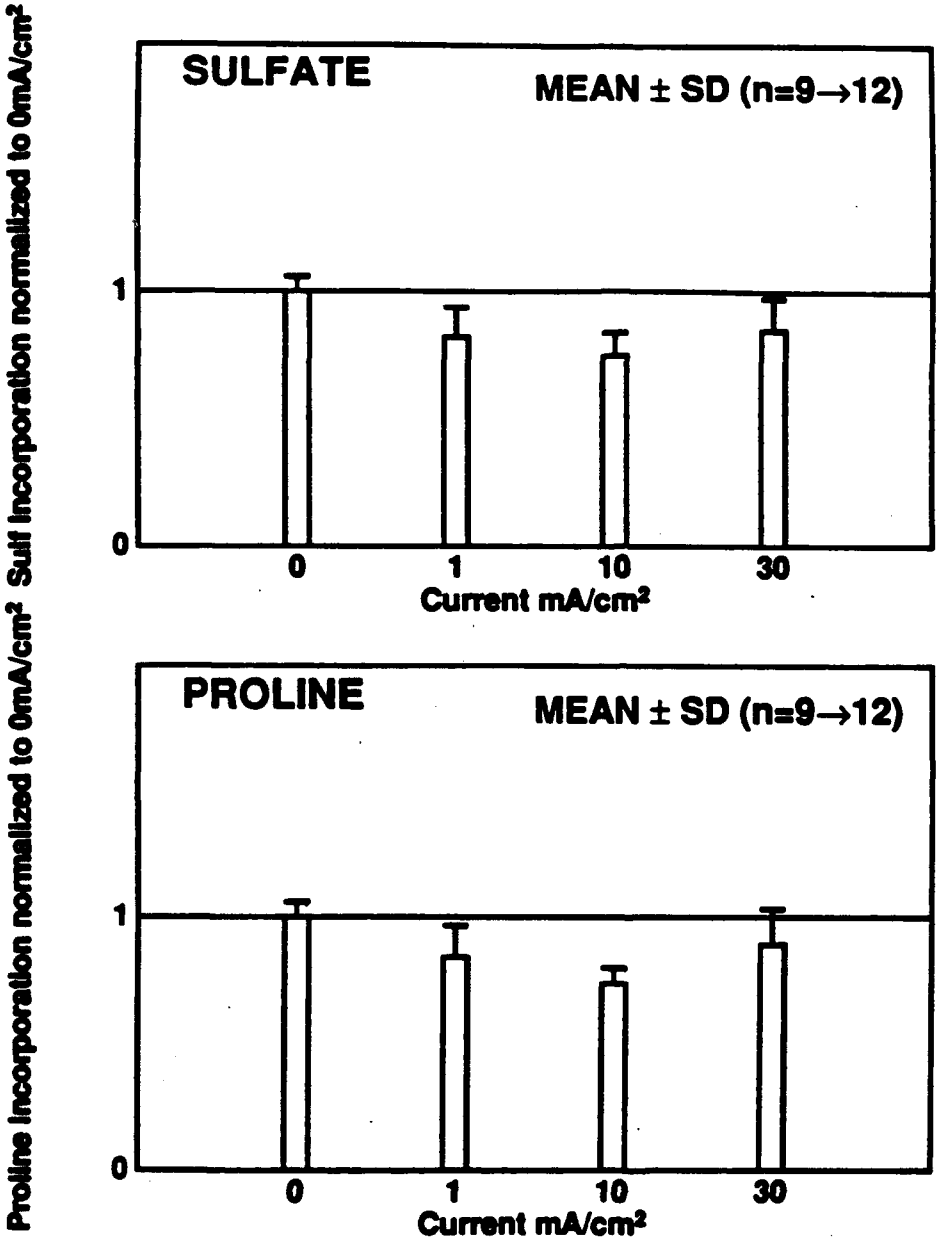


Figure 5 Groups of 12 3mm chondrocyte/agarose disks were subjected to current densities at 100Hz in the presence of radiolabeled sulfate and proline precursors. 2% agarose disks containing chondrocytes were taken on day 6 of the 47 day culture described in Chapter IV and the currents were applied for 24 hours. All the bars at non-zero current densities are significantly different from the control group with no applied current, $p < 0.05$ using the two-tailed t-test.

more or less sensitive to applied electric fields.

Therefore, another set of experiments using Swarm rat chondrosarcoma cells were performed in collaboration with Dr. R. Schneiderman and Prof. J. H. Kimura at Rush-Presbyterian St. Luke's Medical Center, Chicago. (Prof. Kimura is one of the pioneers in the use of this continuous cell line for the study of proteoglycan biosynthesis by chondrocytes.) Swarm rat chondrosarcoma cells from continuous monolayer were harvested and incorporated into 2% agarose in the electrical exposure chamber shown schematically in Fig. 6. The cells were incubated for 24 hours in the chamber and then subjected to graded levels of sinusoidal current densities at a frequency of 1 Hz. Proteoglycans were subsequently extracted from the gels using 4M GuHCl, and the macromolecular radioactivity was separated from unincorporated isotope via Sephadex G-25 chromatography.

Fig. 7 shows the radioactivity in the test gels normalized to that in the unstimulated controls. There was no significant difference between test and control specimens for current densities less than 0.3 mA/cm^2. However, for currents between 0.3 and 2.0 mA/cm^2, a 10-25% increase in proteoglycan synthesis was observed. 2.0 mA/cm^2 current densities at frequencies of 0.1, 10, 100, and 10^4 also appeared to stimulate synthesis. These results suggested that a a stimulation of proteoglycan biosynthesis could be observed in this homogeneous cell population at lower current densities than that seen in fresh bovine cells (Figures 3 and 4). Furthermore, these chondrosarcoma cells appeared to ber capable of modulating their biosynthetic pattern at such lower current densities even in the absence of an appreciable extracellular matrix.

CONCLUSIONS

The results of these studies suggest that: (1) both normal chondrocytes and Swarm rat chondrosarcoma cells in agarose culture can, under proper culture conditions, continue to synthesize matrix macromolecules at a rate similar to that in native

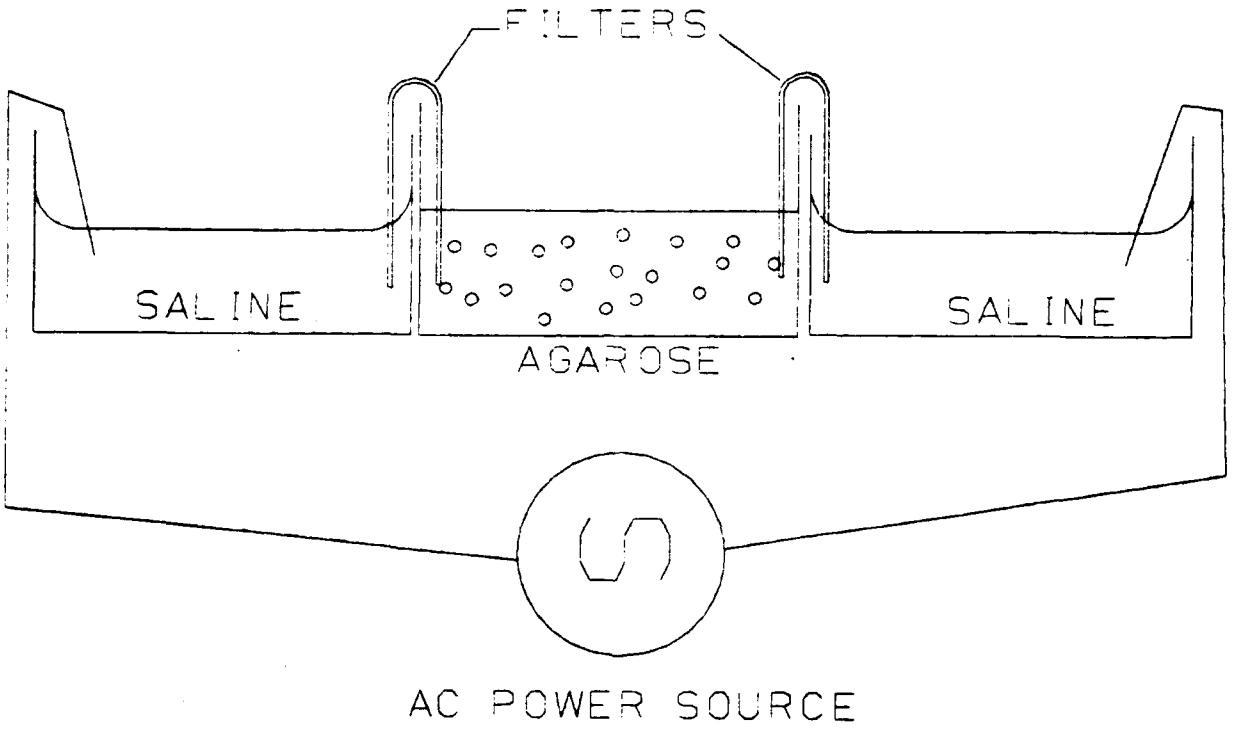
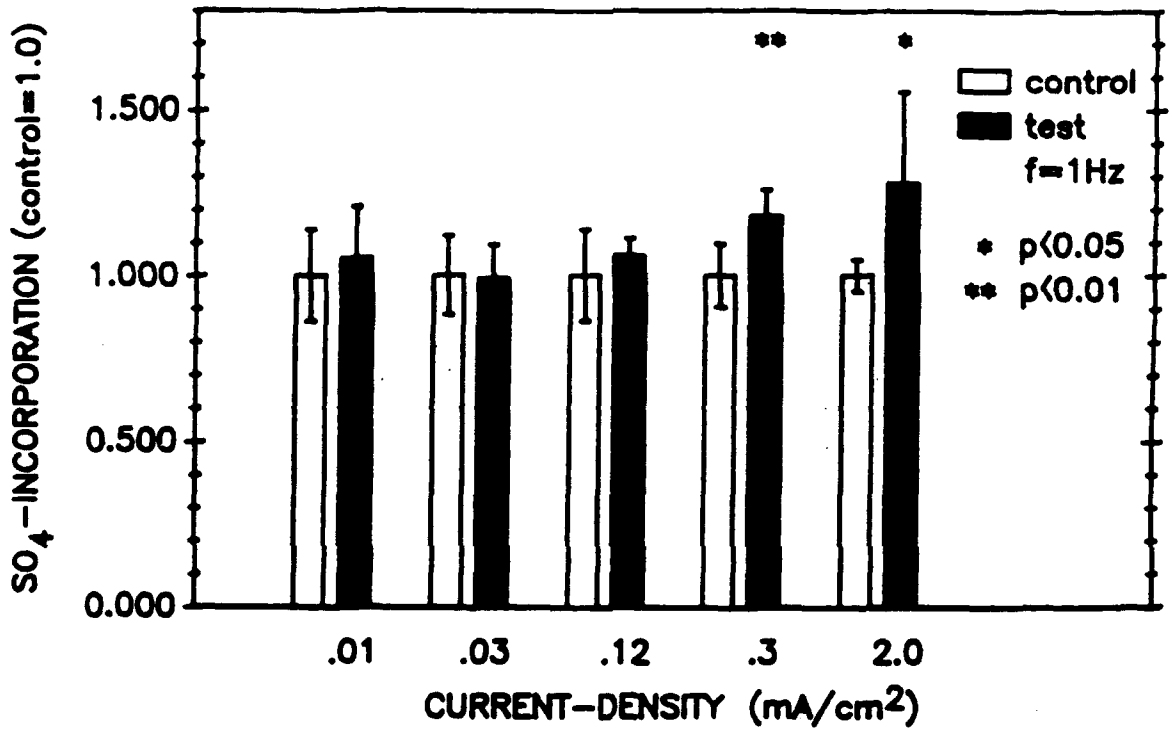


Figure 6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CURRENT-DENSITY (mA/cm2)

Figure 7

cartilage, and (2) electric fields applied to chondrocytes in agarose can modulate the synthesis of proteoglycans and protein constituents. Biosynthesis may be inhibited or stimulated depending on the amplitude of the applied current density. In addition, the presence of extracellular matrix may enhance the ability of normal chondrocytes and cells in intact cartilage to respond to electric fields, although the presence of matrix was not required for the stimulatory response to be observed with Swarm rat chondrosarcoma cells.

These results also provide support for the long-term objectives of this research in that the chondrocyte/agarose system appears to be an appropriate model with which to study the mechanisms by which physical forces (e.g., electrical and mechanical) may interact with mammalian cells. The chondrocytes can be maintained in a natural environment which, in certain ways, is more amenable than that of intact cartilage to the study of mechanism at the single cell level.

PUBLICATIONS

The following publications have appeared, been submitted, or are in preparation, based on research supported by AFOSR Grant 91-0153, and are included as Appendices:

(1) Buschmann, M.D., Gluzband, Y.A., Grodzinsky, A.J., Kimura, J.H., and Hunziker, E.B., "Chondrocytes in Agarose Culture Synthesize a Mechanically Functional Extracellular Matrix," J. Orthopaedic Research, vol. 10, 1992 (in press).

(2) Buschmann, M.D. and Grodzinsky, A.J., "A Molecular Model of Proteoglycan-Associated Electrostatic Forces in Cartilage Mechanics," in preparation for submission to the J. Biomechanical Engineering.

PERSONNEL

Professor Alan J. Grodzinsky, Principal Investigator
Michael D. Buschmann, Graduate Research Assistant
Yehezkiel A. Gluzband, Technical Research Specialist

Advanced Degrees Awarded:

Ph.D. to Michael D. Buschmann, February, 1992, for a Ph.D. Thesis entitled, "Chondrocytes in Agarose Culture."

Conference Presentations:

Parts of the research described in this report were presented at the Symposium on Physical Control of Cellular Response during the 38th Orthopaedic Research Society Meeting, February 17-20, 1992, Washington, DC.

REFERENCES

1. Osepchuck, J.M. ed., Biological Effects of Electromagnetic Radiation, IEEE Press, New York, 1983.

2. Weaver, J.C. and Astumian, R.D., "The response of Living Cells to Weak Electric Fields: The Thermal Noise Limit," Science, 247:459-462, 1990.

3. Benya, P.D. and Shaffer, J.D., Dedifferentiated Chondrocytes Reexpress the differentiated Collagen Phenotype When Cultured in Agarose Gels, Cell, 30:215-224, 1982.

4. Frank, E.H., and Grodzinsky, A.J., Cartilage Electromechanics I: Electrokinetic Transduction and the Effects of Electrolyte pH and Ionic Strength, J. Biomechanics, 20:615-627, 1987.

5. Frank, E.H., and Grodzinsky, A.J., Cartilage Electromechanics II: A Continuum Model of Cartilage Electrokinetics and Correlation with Experiments, J. Biomechanics, 20:629-639, 1987.

6. MacGinitie, L.A., Grodzinsky, A.J., Frank, E.H., and Gluzband, Y.A., Frequency and Amplitude Dependence of Electric Field Interactions: Electrokinetics and Biosynthesis, in Mechanistic Approaches to Interactions of Electromagnetic Fields with Living Systems, M. Blank and E. Findl, eds., Plenum Pub., New York, 1987.

7. MacGinitie, L.A., and Grodzinsky, A.J., Physiologically Generated and Externally Induced Fields: Contrasting Forces and Flows, 7th Proceedings IEEE Engineering in Medicine and Biology Society, 7:367-370, 1985.

8. Grodzinsky, A.J. and Nussbaum, J.H., Theoretical Models For Interaction of Electromagnetic Fields with Biological Tissues, Final Report under USAFSAM Contract A-3529-230-S1, June, 1987.

9. Grimshaw, P.E., Grodzinsky, A.J., Yarmush, M.L. and Yarmush, D.M., "Dynamic Membranes For Protein Transport: Chemical and Electrical Control," Chem. Eng. Sci., 44:827-840, 1989.

10. Grimshaw, P.E., Grodzinsky, A.J., and Yarmush, M.L., Selective Augmentation of Macromolecular Transport in Gel Membranes by Electrodiffusion and Electrokinetics," Chem. Eng. Sci., 45:2917-2929, 1990.

11 Grimshaw, P.E., Nussbaum, J.H., Grodzinsky, A.J., and Yarmush, M.L., "Kinetics of Electrically and Chemically Induced Swelling in Polyelectrolyte Gels," J. Chemical Physics, 93:4462-4472, 1990.

12. Adey, W. R., and Lawrence, A.F., eds., Nonlinear Electrodynamics in Biological Systems, Plenum Press, New York, 1984

13. Mankin, H. J. and Brandt, K. D., Biochemistry and Metabolism of Cartilage in Osteoarthritis, in Osteoarthritis, Moskowitz, R. W., Howell, D.S., Goldberg, V.M., and Mankin, H.J., Eds., W. B. Saunders, Phila., 1984, 43.

14. Hay, E.D., ed., Cell Biology of Extracellular Matrix, Plenum Press, New York, 1981.

15. Schneiderman, R., Keret, D., and Maroudas, A., Effects of Mechanical and Osmotic Pressure on the Rate of Glycoasminoglycan synthesis in Human Adult Femoral Head Cartilage, J. Orthopaedic Research, 4:393-408, 1986.

16. Sah, R.L., Kim, Y.J., Doong, J.Y., Grodzinsky, A.J., Plaas, A.H.K., and Sandy, J.D., "Biosynthetic Response of Cartilage Explants to Dynamic Compression," J. Orthopaedic Res., 7:619-636, 1989.

17. Gray, M.L., Pizzanelli, A.M., Grodzinsky, A.J., and Lee, R.C., Mechanical and Physicochemical Determinants of the Chondrocyte Biosynthetic Response, J. Orthop. Res., 6:777-792, 1988.

18. Frank, E. H., Grodzinsky, A.J., Koob, T. J., and Eyre, D.R., Streaming Potentials: A Sensitive Index of Enzymatic Degradation in Articular Cartilage, J. Orthopaedic Research, 5:497-508, 1987.

19. Sachs, J.R. and Grodzinsky, A.J., "A Mathematical Model of an Electromechanically Coupled Poroelastic Medium Driven by an Electric Current," Physico-Chemical Hydrodynamics, 11:585-614, 1989.

20. Eisenberg, S.R. and Grodzinsky, A.J., "Electrokinetic Micromodel of Extracellular Matrix and Other Polyelectrolyte Networks," PhysicoChemical Hydrodynamics, 10:517-539, 1988.

21. Fukada, E., Inoue, S., Sakau, T., Takahashi, H., and Tsuyama, N., eds., Bioelectrical Repair and Growth, Nishimura Ltd., Niigata, Japan, 1985.

22. MacGinitie, L.A., Grodzinsky, A.J., Gluzband, Y.A., and Varshavsky, A., Protein Synthesis in Cartilage in Response to Applied Electric Fields, 9th Bioelectromagnetics Society, 16, 1987.

23. MacGinitie, L.A., Gluzband, Y.A and Grodzinsky, A.J., "Electric Field Modulation of Protein Synthesis in Articular Cartilage Organ Culture," (submitted).

24. Sun, D., Aydelotte, M.B., Maldonado, B., Kuettner, K.E., and Kimura, J.H., Clonal Analysis of the Population of Chondrocytes from the Swarm Rat Chondrosarcoma in Agarose Culture, J. Orthopaedic Research, 4:427-436, 1086.

25. Aydelotte, M.B., Greenhill, R.R. and Kuettner, K.E., "Differences Between Sub-Populations of Cultured Bovine Articular Chondrocytes. II Proteoglycan Metabolism," Conn. Tis. Res., 18:223-234, 1988.

26. Benya, P.D., Brown, P.D., and Padilla, S.R., "Microfilament Modification by Dihydrocytochalasin B Causes Retinoic Acid-Modulated Chondrocytes to Re-

express the Differentiated Collagen Phenotype Without a Change in Shape," J. Cell Biol., 106:161-170, 1988.

27. Kuettner, K.E., Memoli, V.A., Pauli, B.U., Wrobel, N.C., Thonar, E.J.-M.A., and Daniel, J.C., J. Cell Biol., 93, 751-757, 1982.

28. Farndale, R.W., Buttle, D.J., and Barrett, A.J., "Improved Quantitation and Discrimination of Sulphated Glycosaminoglycans by Use of Dimethylmethylene Blue," Biochim. Biophys. Acta, 883:173-177, 1986.

29. Kim, Y-J., Sah, R.L., Doong, J-Y.H., and Grodzinsky, A.J., "Fluorometric Assay of DNA in Cartilage Explants Using Hoechst 33258," Analytical Biochemistry, 174:168-176, 1988.

30. Sah, R.L-Y., Sc.D. Thesis, MIT, 1990.

31. Tiao, P., S.B. Thesis, MIT, 1989.